target molecule is in the presence of a test molecule allows detection and quantification of binding between the test molecule and the target molecule(s).

[0054] Another optional method of detecting changes in conformation of molecules in the invention is by measurement of dielectric properties. As molecules bind and undergo conformational changes, their dielectric properties change as well. These changes can be quantified and used to determine molecular interaction parameters.

[0055] An optional way of detecting changes in conformation of the target molecules being assayed in the current invention is through calorimetric measurement. Changes in heat capacity are measured as the molecules in the assay undergo temperature induced denaturation. As with the fluorescence method, binding between molecules is detected and quantified through comparison of the thermal property curves from assays done with just the target molecule and assays done with the combination of the target molecule and test molecule(s).

[0056] Thermal Property Curves

[0057] The unfolding, disassociation or denaturing of a target molecule(s) in response to changes in temperature can be useful in many applications, e.g., in determining the stability of a specific protein under specified conditions, or in the identification of a nucleic acid, the detection of SNPs in a nucleic acid, etc. The measurement of the molecular denaturing, disassociation or unfolding of the target molecule is used to construct a thermal property curve. In other applications, variations of basic thermal property curves can be used to test for, e.g., whether a specific ligand or other molecule binds to a target molecule. For example, the binding of a specific ligand to a specific receptor can be investigated by a thermal property curve. See, e.g., Gonzalez, M. et al., (1997) "Interaction of Biotin with Streptavidin" *J Biol Chem,* 272(17): 11288-11294. Additionally, hybridization of specific oligonucleotides to each other can be demonstrated with a thermal property curve. See, e.g., Clegg, R. et al. (1994) "Observing the helical geometry of double-stranded DNA in solution by fluorescence resonance energy transfer." *Proc Natl Acad Sci USA* 90(7):2994-2998.

[0058] Stabilization Due to Binding

[0059] Thermal property curves are based on the change in conformation of a molecule due to changes in temperature. As stated above, molecules, e.g., proteins and nucleic acids, show unfolding, disassociation or denaturation over a range of temperatures. The measurement of the unfolding, etc. of a given target molecule as a function of temperature generates a thermal property curve for that molecule. Binding of, e.g., ligands (e.g., such as nucleic acids or protein) to the target molecule, can lead to stabilization of the molecule and hence a change in its thermal property curve. See, e.g., Gonzalez, M. et al., (1997) "Interaction of Biotin with Streptavidin" *J Biol Chem,* 272(17): 1.1288-11294; Schellman, J. (1975) *Biopolymers,* 14:999-1018; Barcelo, F. et al., (1990) "A scanning calorimetric study of natural DNA and antitumoral anthracycline antibiotic-DNA complexes." *Chem Biol Interactions,* 74(3):315-324; Schellman, J. (1976) Biopolymers, 15:999-1000; and Brandts, J. et al. (1990) "Study of strong to ultratight protein interactions using differential scanning calorimetry" *Biochem* 29:6927-2940.

[0060] In other words, binding of the test molecule to the target molecule, e.g., ligand will cause the target molecule to denature (or disassociate, unfold, etc.) in a different manner than it would without the binding. This property, of course, can be extremely useful in many applications, e.g., determining the relative binding affinities of multiple ligands to a target molecule or the binding abilities of mutant proteins, e.g., as described herein. In the generation of thermal property curves, "T_m," denotes the "midpoint temperature" or the temperature at which the denaturation or unfolding reaction is half complete.

[0061] In some aspects of the current invention, construction of thermal property curves similar to those discussed above can be used to determine a reference temperature encountered by a solution in a microfluidic channel. More specifically, embodiments of the invention allow a reference temperature of a fluid within a microfluidic channel to be correlated to a value of a physical parameter outside the channel that can be readily measured. For many applications, e.g., PCR and/or construction of thermal property curves to test for, e.g., binding of ligands, etc., precise temperature control is needed within the microfluidic elements, such as channels or chambers, of microfluidic devices. The use of a melting curve generated for known molecules (e.g., streptavidin/biotin-fluorescein, etc.) to monitor and calibrate the temperature within microfluidic elements is of great benefit since, e.g., it allows for determination of temperature through use of simple, inexpensive materials, the process can be substantially irreversible or optionally reversible (see, below), it can be fine-tuned by use of different molecules having different melting temperatures (see, below), and it eliminates the need to place a sensor within the microfluidic device. The physical parameter to which the reference temperature is correlated must be a physical parameter that correlates to the temperature within the channel. For example, the physical parameter could be the temperature at an exterior surface of the microfluidic device adjacent to the microfluidic element, the electric current applied to fluid in the element that joule heats the fluid, or the temperature of a thermal block in thermal contact with the microfluidic element.

[0062] In some optional embodiments of the present invention, molecules such as streptavidin and biotin-fluorescein are used to calibrate the temperature in a microfluidic device by determining a reference temperature. Free streptavidin (SA), which has a melting temperature of approximately 74° C., is optionally bound with four molecules (e.g., of biotin-fluorescein, etc.), thus causing the SA to have a very sharp melting temperature of about 108° C. When bound to streptavidin, the fluorescein is substantially quenched (i.e., it does not emit fluorescence). But, when the streptavidin molecule is denatured (i.e., by heat in the microchannel), the biotin-fluorescein conjugate is released and thus fluorescence can be detected. To bind biotin-fluorescein to SA, the SA is optionally incubated in the presence of excess biotin-fluorescein conjugate. After saturation, unbound biotin-fluorescein is removed by, e.g., size filtration. The melting temperature of this SA-biotin-fluorescein complex is determined on, e.g., a heated fluorometer or other comparable detector. The SA-biotin-fluorescein complex is then optionally passed through the microchannels of a device of the current invention (i.e., which have a targeted reference temperature). If the proper fluorescence from the SA-biotin-fluorescein system is detected at a location along the length of the microchannel (or at another convenient location) that is programmed to be at the reference temperature, then the predetermined reference temperature was attained in the channel. If the proper fluorescein fluorescence is not detected, then the proper reference temperature was not achieved in the microchannel. In various